

A STUDY OF THE EFFECTS OF MODIFICATIONS
OF THE CULTURE MEDIUM UPON LENGTH OF
LIFE AND FECUNDITY IN A ROTIFER, PROALES
SORDIDA, WITH SPECIAL RELATION TO THEIR
HERITABILITY

RUTH STOCKING LYNCH
HELEN BERENICE SMITH

A DISSERTATION

SUBMITTED BY THE JUNIOR AUTHOR TO THE BOARD OF UNIVERSITY
STUDIES OF THE JOHNS HOPKINS UNIVERSITY IN CONFORMITY
WITH THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

BALTIMORE
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RUTH STOCKING LYNCH AND HELEN BERENICE SMITH

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INTRODUCTION

Extensive investigations on the Infusoria have demonstrated that these organisms, while undergoing continuous vegetative reproduction, under certain conditions "run down,"—become depressed. In many of the earlier genetic studies this progressive depression was interpreted to mean that the organisms have a definite life cycle with periods of youth, maturity, and old age, representing through the course of many consecutive generations of vegetative reproduction the usual life cycle of a single metazoan organism. The results of later investigators, however, show that in many cases the depression is partly or entirely due to unfavorable environmental conditions. Enriques, Woodruff, and others found that under adequate cultural conditions certain Infusoria can be cultivated indefinitely without decline in vitality, although the same Infusoria under poor cultural conditions exhibit a depression which may continue for long periods, becoming more and more marked as generations pass and eventually resulting in the death of most of the lines.

The inheritance of such a depression, after restoration to normal conditions, was tested by Middleton (1918) and by Jollos (1921). Middleton found that a depressed condition induced in *Stylonchia pustulata* by long continued culture at a high temperature is inherited for at least twenty days after restoration to normal temperature. Jollos produced a depression of the fission rate in *Paramecium aurelia* by subjection to calcium nitrate; he found that this lowered rate of reproduction is heritable for four months. In most other cases it has not been possible to test critically the inheritance of the depression after restoration to normal conditions, but it is commonly assumed that inheritance occurs. (For a detailed review of this type of work on the Protozoa, see Jennings, 1929.)

The present investigation was undertaken to determine how far

conditions in the lower Metazoa are comparable to those in the Infusoria, particularly as to the production of depression, its increase as generations pass, and its inheritance after return to normal conditions. The Rotifera are particularly suitable for such an investigation. They resemble the Infusoria in size, habitat, and mode of life, but reproduce by means of germ cells which undergo a process of development in the production of the many-celled individual. They can be subjected to the same conditions that induce depression in the Infusoria, and the depression so produced can be studied in relation to its tendency to cumulation and its persistence after return to normal conditions.

The particular species of rotifer chosen, *Proales sordida* Gosse, has the additional advantage of reproducing exclusively by parthenogenesis, that is, from single parents, as do the Protozoa in vegetative reproduction. The complications of biparental inheritance, common to most types of germ-cell reproduction, are therefore avoided.

Insufficient feeding was the method used for producing depression: certain lines were well-fed; other lines were given insufficient food in definite ratios to the amount given the well-fed group. This method is the one used by Beers (1926) with the infusorian *Didinium*. He found that his well-fed group exhibited a high fission rate throughout his experiment; and that his inadequately-fed group showed from the first a lower fission rate, which after some time became still further depressed, this depression steadily increasing until the line encysted.

Previous experimental work on the Rotifera has been concerned chiefly with the problem of sex-determination; but there are a few papers which bear on the questions here considered. Among these are four on the effects of food control during parthenogenetic reproduction. Luntz (1926), in a critical study of the relation of food to the onset of the sexual generation in *Pterodina elliptica*, describes briefly an experiment on quantitative variation of food. Using Beneke's solution as the culture medium, and *Polytoma* or *Chlamydomonas* as the food, he varied the amount of available food by varying the number of drops used. He found that the number of offspring produced varied directly and precisely with the amount of food provided. He does not give data for successive generations; this work therefore furnishes no data on cumulative effects.

Shull (1912) and Whitney (1912a) carried on extensive investigations bearing upon this matter, in successive generations of the rotifer *Hydatina senta*. Both of these investigators, using various types of culture media, found that races reproducing exclusively by parthenogenesis decline in vitality, as indicated by their lowered fecundity. Whitney suggests that this may be due to "the constant environment

of the horse manure cultures" (1912a, p. 343). Lambert, Rice, and Walker (1923), working under the direction of Whitney, show that this decline in vitality is due to the unbalanced diet, not to the parthenogenetic method of reproduction. None of these four investigations take up the question of the heritability of these depressions.

Other investigations, in which alcohol is used as the modifying factor, do deal with the heritability of modifications produced. Whitney (1912c) subjected *Hydatina senta* to alcohol and produced a decrease in fecundity which continued for two generations after return to a normal culture medium, but not longer. Noyes (1922) with *Proales decipiens*, and Finesinger (1926) with *Distyla inermis*, produced a cumulative decline in fecundity by subjection to alcohol and found that on return to normal conditions the depression continued to some degree for two generations only. (Finesinger, however, found that in weak doses alcohol acts as a stimulant and causes a slight increase in both fecundity and longevity.)

In the present study, dealing with the effects of a reduced food supply, two main questions are considered: First, do adverse environmental conditions produce the same effects in the Rotifera as in the Infusoria; that is, do they bring about a decline which increases with succeeding generations, so that the Rotifera run down as do the Infusoria? Second, if they become depressed, is this depression inherited in succeeding generations when the progeny are returned to a favorable environment?

Two characteristics, fecundity and length of life, were taken as indices of vigor. The effect of the reduced food supply on these indices was studied during the summer of 1928; the results of these experiments are presented in Part I. During the summer of 1929 the experiments, somewhat modified, were repeated; their results are presented in Part II. All of the experimental work was done in the Marine Biological Laboratory at Woods Hole.

The investigation was undertaken at the suggestion of Professor H. S. Jennings. It gives us great pleasure to express our appreciation of his advice throughout the investigation and his help in the preparation of the manuscript.

THE ORGANISM

Proales sordida is a rotifer well suited to this type of investigation. It lives in ponds and sluggish streams, and can be cultivated with ease in the laboratory in almost any infusion favorable to the growth of Protozoa; it feeds on bacteria and other minute organisms. The usual length of life is eight days, though occasionally individuals live as long as twenty-five days. Each animal produces twenty-four to twenty-

eight eggs, with an observed maximum of thirty-four eggs. The adults average 300 microns in length. Reproduction is exclusively parthenogenetic. The species consists entirely of females; no males have ever been described and none have appeared during the history of our cultures even under conditions known to be favorable to male production in other rotifers. It was therefore possible, by starting with a single ancestor, to have all the animals used in these experiments members of a single clone, forming a group as genetically similar as it is possible to obtain. (For a more detailed description of this organism see Jennings and Lynch, 1928, I.)

METHODS

It is not only necessary, for this type of experimentation, to have animals that are alike genetically; it is also necessary to have them as uniform as possible in other respects. Uniformity in age is particularly important, since Jennings and Lynch (1928, I) have demonstrated that the offspring of young parents have a lower fecundity than the offspring of the same parents when old. *Proales sordida* has an average life of only eight days; it begins to lay eggs about forty-eight hours after hatching. Its eggs, laid in the one-cell stage, take from twenty to twenty-four hours to hatch. A population uniform in regard to age may therefore be obtained by including in it only such eggs as are laid within a five-hour period by parents differing in age by less than twenty-four hours. To meet these conditions the following procedure was employed: In starting each experiment, several thousand eggs were removed from mass cultures and placed in oatmeal infusion in culture dishes, three hundred to a dish. Twenty-four hours later a small amount of infusion was added to each dish; at that time most of the eggs had hatched. After the lapse of another twenty-four hours, when most of the animals had begun to lay eggs, the animals were transferred to dishes of fresh culture medium. The eggs laid within the next five hours were removed and placed on two-concavity slides, one egg in each concavity in a single drop of culture medium. The animals hatching from these eggs were the first generation of experimental animals. By this procedure, eggs of approximately the same age were obtained from parents of about the same age, thus fulfilling the requirements outlined above. Throughout the remainder of the investigation, except in rare cases to be mentioned at the appropriate places, no eggs later than the third were used to give rise to a new generation.

Without exception the animals were cultivated individually on ground-glass, two-concavity slides in single drops of culture medium. The slides were kept on glass supports in nine-inch crystallizing dishes, twelve slides to a dish. Each dish was water-sealed by placing boiled

spring water in the bottom of the larger portion and inverting over it the smaller portion.

The animals were examined at approximately twelve-hour intervals. In the morning each animal was transferred by means of a capillary pipette to a fresh drop of culture medium on a clean slide. In the evening the eggs which had been laid during the day were removed from the drops, but the animals were left undisturbed. The capillary pipettes were sterilized in boiling water before each transfer. Pipettes of the same size were used to place the culture fluid on the slides. The pipettes were boiled daily in distilled water and the rubber bulbs were boiled at frequent intervals.

A favorable culture medium for *Proales* is an oat infusion prepared by boiling fifteen flattened flakes ($\frac{1}{4}$ gram) of rolled oats ("Quaker Oats") for three minutes in 100 cc. of spring water, filtering this, and allowing it to stand for twenty-four hours before using (*cf.* Jennings and Lynch, 1928, I). The strength of this culture fluid may be easily varied by changing the number of flakes of oatmeal used. Such varied solutions were used in these experiments. In every case the media were prepared simultaneously in the same way, the only difference between them being the number of flakes of oatmeal used.

PART I. EXPERIMENTS OF 1928

In this series of experiments the following strengths of culture medium were used:

Control Series	C, full-strength	fluid, 15 flakes oatmeal to 100 cc. spring water
Test	" H, half-strength	" 8 " " " " "
"	" Q, quarter-strength	" 4 " " " " "
"	" S, boiled spring water only	

These media were prepared as described above and were left exposed to the air for twelve hours before being corked.

In the preliminary experiments it was found that the animals would not live beyond the second generation in boiled spring water without oatmeal (S culture medium), nor in the four-flake solution (Q culture medium).

In the main experiment, begun July 19, 1928, and continued for three months, three groups of animals were studied: one group (C) was cultivated in full-strength culture medium; a second (H) in half-strength culture medium; and a third (Q) was put for its first generation into quarter-strength culture medium, into full-strength for its second generation, and then again into quarter-strength culture medium, where it remained until its death. Some individuals of the later genera-

tions of both the *H* and the *Q* groups, and their descendants, were cultivated in full strength medium (*HC* and *QC* groups).

At the start, one hundred individuals in each generation of all three groups were kept throughout their lives. But the rapid increase in co-existing generations so increased the number of animals to be examined daily that a change in procedure was necessary. In most cases the number of individuals to a generation was reduced, usually to about fifty (*cf.* Tables I and II). In other cases, not only were the members of a given generation reduced in number, but they were kept just long enough to produce the next generation, and then discarded. Such generations, of course, yielded no data on fecundity or longevity.

TABLE I

Experiment I, 1928—Showing for the successive generations (Gen.) of the two control series *C* and *M* the total numbers of eggs isolated (No.); the numbers of these that did not hatch (*N.H.*), and the numbers that hatched (*H.*); and for the latter the mean numbers of eggs laid and the mean numbers of days lived. Data presented graphically in Figs. 1 and 2.

C Series (full-strength medium)						M Series (full-strength medium)					
Gen.	No.	N. H.	H.	Mean Eggs	Mean Days	Gen.	No.	N. H.	H.	Mean Eggs	Mean Days
1	92	1	91	7.67	5.76	1	49	1	48	3.60	5.23
2	106	5	101	6.02	4.85	2	95	7	88	1.93	4.32
3	47	6	41	6.41	5.02	3	51	16	35	7.88	7.02
4	52	7	45	6.22	4.77	4	50	2	48	11.31	9.56
5	57	3	54	6.18	4.49						
6	58	8	50	3.02	3.74	9	49	1	48	9.31	11.61
7	49	8	41	1.95	3.68						
8	52	3	49	2.55	4.11	11	49	0	49	8.85	9.45
9	75	17	58	1.77	4.00						
10	56	6	50	2.74	4.18	13	42	1	41	11.58	9.17
11	51	7	44	2.40	4.38						
12	60	12	48	2.04	3.51						
13	49	4	45	1.73	3.18						
14	48	3	45	1.66	3.75						
15	49	5	44	1.36	4.15						
16	40	7	33	1.81	4.59						
17	40	4	36	2.16	4.56						
18	45	6	39	5.41	6.25						
19	49	4	45	12.17	10.06						
24	51	1	50	8.86	10.70						
26	39	1	38	12.57	10.71						
28	44	0	44	16.52	11.44						

TABLE II

Experiment I, 1928. Showing for the successive generations (Gen.) of the *H* series (cultivated in half-strength medium), the *Q* series (cultivated in quarter-strength medium), and the *HC* and *QC* series (cultivated in full-strength medium), the total numbers of eggs isolated (No.), the numbers of these that did not hatch (*N.H.*), and the numbers that hatched (*H.*); and for the latter the mean numbers of eggs laid and the mean numbers of days lived.

H Series (half-strength medium)						Q Series (quarter-strength medium)					
Gen.	No.	N. H.	H.	Mean Eggs	Mean Days	Gen.	No.	N. H.	H.	Mean Eggs	Mean Days
1	97	1	96	2.16	3.69	1	100	0	100	0.18	2.01
2	93	0	93	2.19	3.69	†(2)	17	0	17	7.76	5.50
3	87	6	81	2.54	3.16	3	55	8	47	0.95	1.96
4	52	10	42	3.97	3.78	*4	17	0	17	2.00	3.52
5	46	2	44	3.43	3.95	5	34	2	32	1.68	3.09
6	62	0	62	2.41	3.64	6	49	1	48	1.02	2.70
7	45	3	42	2.11	3.42	7	49	0	49	0.48	2.53
8	45	4	41	1.24	2.85	*8	13	1	12	0.00	1.75
9	43	6	37	0.94	2.75	QC Series (full-strength medium)					
10	18	2	16	0.62	2.50						
*11	7	0	7	0.14	3.00	*1(8)	11	2	9	4.00	4.72
*12	10	0	10	0.50	2.25	*2(9)	33	6	27	1.29	3.77
*13	4	1	3	0.00	1.83	3(10)	34	4	30	1.30	3.30
HC Series (full-strength medium)						*4(11)	35	13	22	0.90	2.95
*1(10)	14	2	12	2.50	3.95	*5(12)	20	3	17	0.64	3.26
*2(11)	27	4	23	1.04	3.19	*6(13)	9	0	9	0.77	3.88
*3(12)	23	7	16	1.62	2.90	*7(14)	3	0	3	2.00	4.33
*4(13)	24	3	21	2.90	4.57	*8(15)	2	0	2	5.50	7.75
5(14)	48	1	47	1.91	4.25	*9(16)	8	0	8	8.12	9.18
6(15)	58	10	48	2.14	4.37	10(17)	44	4	40	5.50	7.80
7(16)	46	6	40	2.47	4.72	11(18)	46	3	43	6.06	8.38
8(17)	45	5	40	6.45	6.83	12(19)	47	0	47	5.48	8.70
9(18)	49	7	42	9.90	8.61	13(20)	54	0	54	6.98	10.24
14(23)	41	2	39	8.07	11.14	15(22)	47	0	47	9.68	9.95
16(25)	39	0	39	10.38	10.23	17(24)	50	0	50	14.34	11.01
18(27)	49	0	49	15.14	11.15						

†(2) Grown in full-strength medium.

* Generations comprising less than thirty animals.

*General Description***I. The Control Series.**

During the course of the experiment the control animals lived through twenty-eight generations, showing great variability in length of life and fecundity. This variation appeared to be correlated with the temperature fluctuations. The temperature was high when the experiment began and remained high for sixteen generations; these generations showed a progressive decline in both fecundity and longevity. The succeeding generations (17th to 27th), subjected to a lower temperature, showed a recovery which started with the first generation grown at the cooler temperature. This recovery was so swift, both fecundity and longevity increased so rapidly, that with the 20th generation it became necessary to reduce the number of animals examined. This was done by testing only occasional generations, as noted above. The number of animals in the generations not tested (generations 20, 21, 22, 23, 25, and 27) was re-



FIG. 1. Experiment I, 1928. Comparison of the two control series *M* and *C*, based on egg production; horizontal axis represents successive generations; vertical axis, mean egg production.

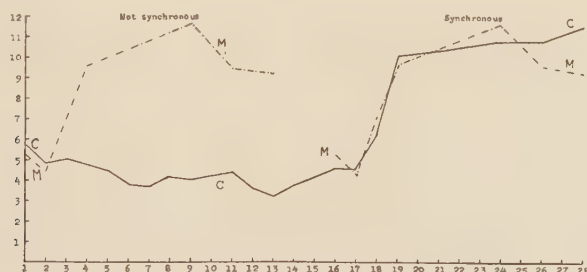


FIG. 2. Experiment I, 1928. Comparison of the two control series *M* and *C*, based on length of life; horizontal axis represents successive generations; vertical axis, mean length of life in days.

duced to thirty, and these were maintained only long enough to provide the next generation.

When the control (*C*) animals were in their most depressed condition (generation 15) a special effort was made to determine the cause of their depression by comparing them with a second series of controls, taken at this time from mass culture. This new control series (termed *M*) was treated in every way like the original control series, so that the only known difference between the two sets was the difference of date of derivation from mass culture, resulting, of course, in a diversity in duration of cultivation on slides. If duration of slide cultivation is the effective cause of depression, the early generations of *M* should not show depression; there should be a wide diversity between their records and the records of the synchronous generations of *C* (16th to 28th generations undergoing slide cultivation). If, on the other hand, some current environmental factor is the effective cause, synchronous generations of the two series should yield like data. Figures 1 and 2 demonstrate the latter condition. *C* and *M* show a similar and synchronous modality. Figure 1 is based on egg production; Fig. 2 on length of life. In both figures the record of the second control series *M* is shown in two ways; first, in relation to the records of the early generations of the *C* series, and second, in relation to the synchronous generations of *C*. It is obvious that the record of the *M* series, comprising thirteen generations, follows closely the record of the generations of the *C* series that were living at the same time; not the record of the early generations of *C*. It is therefore concluded that the cause of the depression in *C* was some feature of the current environment, probably the high temperature; and that duration of slide cultivation had had no depressive effect.

II. The *H* Series.

The series cultivated in half-strength culture medium (*H*) was consistently lower in fecundity and length of life than the controls. Consecutive generations showed some variation, but became progressively depressed, and the series died out in the 13th generation. In Table II are given the records of series *H* for every generation, showing the mean egg production and the mean length of life.

Fortunately the ultimate extinction of the *H* series was anticipated and some eggs of the ninth generation were placed in the full-strength culture medium, thus beginning a group of animals (series *HC*) in which to test recovery from depression. At the end of the experiment the *HC* group had been cultivated continuously for eighteen generations in the full-strength culture medium. It showed complete recovery from the depression (see Table II, *HC*). Generations 19, 20, 21, 22, 24, and 26 were not tested.

III. The *Q* Series.

In the preliminary experiments the animals cultivated in the quarter-strength culture medium had not survived beyond the second generation. The exceedingly low records of the first generation of the *Q* series of the main experiment indicated that they were following a similar course. Consequently, it was thought best to rear the animals of the second generation in full-strength culture medium. The records of this second generation of *Q*, cultivated in the full-strength culture medium, greatly exceeded those of the second generation of *C* animals. But the records of the third generation, cultivated in the quarter-strength culture medium, were again very low, although not as low as those of the first generation. The subsequent generations were grown continuously in the quarter-strength culture medium; they declined consistently and died out in the eighth generation.

A few eggs from the seventh generation were placed in the full-strength culture medium to test the recovery of the *Q* series. This *QC* series was continued in the full-strength solution until the end of the experiment. They improved, but their records on the whole were lower than those of the controls (*C* series) and lower than those of the *HC* series. The generations did not overlap as much in this group as in the other two groups, and at the end of the experiment, when the *H-HC* series was in its 27th generation and the *C* series was in its 28th generation, the *Q-QC* series was only in its 24th generation. Generations 21 and 23 were the only ones not tested. The record for each generation of the *Q-QC* series is given in Table II. Because of the low fecundity of these depressed animals the number of individuals in many generations was small, although all the eggs laid were kept, including in some cases eggs later than the third.

Temperature Variation

During the summer of 1927 the laboratory in which these experiments were conducted had shown fluctuations of less than two degrees (*cf.* Jennings and Lynch, I, p. 369), and it was expected that it would maintain the same constancy during the summer of 1928. Unfortunately this was not the case. The temperature of the Woods Hole region was unusually variable from July until September of that year, and the laboratory showed correspondingly greater fluctuations.

The temperature was read at twelve-hour intervals from a Maximum-minimum thermometer which was suspended above the rotifer cultures. The data thus obtained are presented in Fig. 3, which is designed to show the relation between the temperature fluctuations and the

records of the control animals (*C* series). On the vertical axis the temperature is plotted in degrees centigrade; on the horizontal axis the time is plotted in twelve-hour periods, for the duration of the experiment. The upper, shaded curve (*a*) shows the maximum and minimum records for each twelve-hour period, the upper line showing the maximum, the lower line the minimum. The second curve (*b*) is a simplified form of

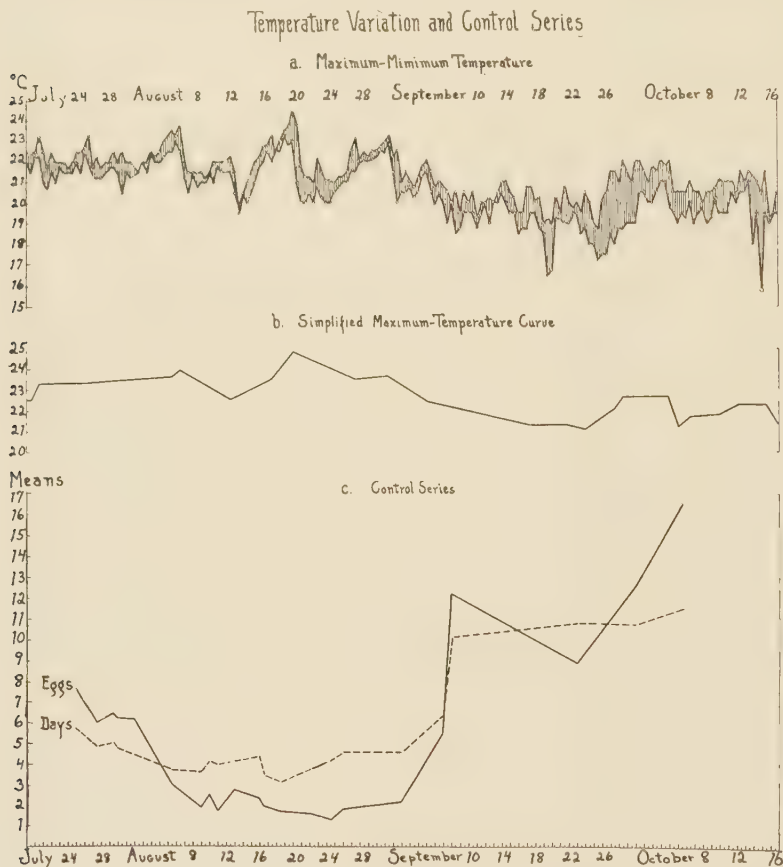


FIG. 3. Experiment I, 1928. Chart showing temperature variation and the records of control series *C*. (*a*) Upper shaded curve: the maximum and minimum temperatures at twelve-hour intervals from July 19th to October 16th; the upper line shows the maximum temperatures; the lower line shows the minimum temperatures. (*b*) Middle curve: a simplified form of the temperature curve giving only the major points of maximum temperature. (*c*) Lower two curves: the record of control series *C* for egg production (solid line) and for length of life (dashed line). The median dates of the twenty-eight generations produced from July 25th to October 5th are indicated on the horizontal axis; the mean number of eggs produced and the mean number of days lived by each generation are indicated on the vertical axis.

the upper line of the shaded chart and shows the highest records of the summer plotted at intervals of approximately one week. The third curve (*c*) shows the records of the control series (*C*) plotted on the same basis as in the foregoing curves. On the vertical scale are plotted the mean number of days of life (dotted-line curve) and the mean number of eggs (solid-line curve) for each generation. On the horizontal scale is recorded the time, as in the upper curves. The records of the controls are plotted by generations on their "Median Dates"; that is, on a date exactly midway between the date when the first animal of a given generation was isolated as an egg and the date when the last animal of that generation died.

A comparison of the three curves (*a*), (*b*), and (*c*), clearly demonstrates a close relation between the records of the control animals and the temperature. As the temperature became higher the animals became more depressed, the lowest records of the controls for the entire experiment being made immediately after the highest temperature of the summer. The animals improved when the temperature became lower. At the end of the summer, when the temperature stayed consistently lower, they gradually recovered from their depression.

Analysis of the Data

I. Method.

Inasmuch as the temperature influenced the results in such a striking way, valid comparisons of the different groups of animals could be made only if the groups to be compared lived at the same time and were thus subjected to the same temperature variations. An arbitrary method was devised to meet this condition. This method was a day by day comparison made on a percentage basis with the control series *C* as the standard.¹ The daily average record of the control series was taken as 100 per cent. The averages for the other cultures on the same day were then expressed as percentages of this. Thus if the average number of eggs produced by *H* on a given day was one-half as great as the number produced by the controls, *H* was given a rating of 50 per cent. In calculating the averages for the controls, the generations as such were disregarded, since, in an adequate medium, the number of generations cultivated on slides had been shown to be unimportant (p. 38). In calculating the records of *H* and *Q*, cultivated in the reduced media, each generation was considered separately.

The procedure for determining the percentage for each generation of an experimental group was as follows, using *H* as an example: First, the mean number of eggs for animals of a given generation of *H* was

¹ This method was devised by Dr. Daniel Raffel of this laboratory.

computed by days on the basis of the day they were begun as individuals. Then this mean for *H* was expressed as a percentage of the mean of *C* for the same day. In this way percentages were calculated for each day

TABLE III

Experiment I, 1928—Showing for the successive generations (Gen.) of the four test series (*H*, *Q*, *HC*, *QC*) the numbers of animals (No.) and the percentages of fecundity and longevity, computed as described in the text (pp. 41–43). Data presented graphically in Figs. 4, 5, 6 and 7.

<i>H</i> Series (half-strength medium)				<i>Q</i> Series (quarter-strength medium)			
Gen.	No.	Fecundity	Longevity	Gen.	No.	Fecundity	Longevity
		<i>per cent</i>	<i>per cent</i>			<i>per cent</i>	<i>per cent</i>
1	96	28.16	64.06	1	100	2.34	34.89
2	93	38.22	75.78	†(2)	17	126.57	108.65
3	81	98.48	75.40	3	47	16.67	41.55
4	42	111.79	84.91	*4	17	40.98	82.81
5	44	85.69	94.54	5	32	70.47	82.68
6	62	80.67	92.01	6	48	55.61	71.99
7	42	115.65	95.60	7	49	35.85	62.92
8	41	69.01	70.67	*8	12	0.00	41.83
9	37	39.34	65.58				
*10	16	24.72	59.22				
*11	7	65.09	74.85				
*12	10	46.88	67.60				
*13	3	0.00	50.15				
<i>HC</i> Series (full-strength medium)				<i>QC</i> Series (full-strength medium)			
*1(10)	12	85.58	89.67	*1(8)	9	140.74	111.46
*2(11)	23	47.75	88.16	*2(9)	27	59.49	93.08
*3(12)	16	104.80	90.83	3(10)	30	83.79	100.47
*4(13)	21	190.96	118.52	*4(11)	22	51.21	78.81
5(14)	47	123.75	98.97	*5(12)	17	42.50	82.52
6(15)	48	115.89	94.68	*6(13)	9	46.99	87.35
7(16)	40	129.71	102.81	*7(14)	3	97.05	91.99
8(17)	40	114.86	110.12	*8(15)	2	261.62	155.04
9(18)	42	80.18	84.93	*9(16)	8	87.20	97.54
				10(17)			
14(23)	39	81.83	111.33	11(18)	No controls for comparison ‡		
				12(19)			
16(25)	39	83.14	95.00	13(20)	54	79.06	93.03
18(27)	49	91.47	97.30	15(22)	47	70.49	92.62
				17(24)	50	86.45	95.91

* Generations comprising less than 30 animals.

†(2) grown in full-strength medium.

‡ Gen. 17, 18, 19 of the *QC* series were begun at a time when no control animals were begun, so comparison on the percentage basis was impossible.

included in that *H* generation. Finally, the percentages were weighted according to the number of *H* animals included in each, and the true mean of these percentages was found for each generation. Following this method, the records of fecundity and longevity of each generation of the experimental groups were calculated as percentages of the synchronous records of the *C* group. The variation of these percentages with the passing of generations is shown graphically in Fig. 4; here the vertical axis represents the percentages, the horizontal axis represents the generations, and the straight horizontal line at 100 per cent represents the standard of comparison,—the record of the *C* group.

A dependable interpretation of the data must take into consideration the numbers of animals involved; unless this number is fairly large, the sample may not be representative. In this study, generations comprising less than thirty animals have been considered of doubtful significance. These generations are indicated by asterisks in Table III. The small numbers in the later declining generations of *H* and *Q* were unavoidable, since the fecundity of the depressed animals was very much reduced and the rate of mortality was high. Fortunately, the records of these later generations are relatively unimportant, as depression is clearly demonstrated in the preceding, larger generations. But in the test of the heritability of depression in the first *HC* and *QC* generations, the small numbers available constitute a serious complication and must be taken into account fully in the interpretation.

II. Depression: (a) Fecundity.

A comparison of the fecundity of the three groups of animals is presented in Fig. 4. The numbers of animals in the various generations of the *H* and *Q* series and their fecundity percentages, computed as described above, are given in Table III.

Both the *H* and *Q* groups showed some variation, but in general they had a much reduced fecundity. The egg production of the first generation of the *H* series was very low; approximately 25 per cent of the *C* average. In the next six generations there was an improvement; the fecundity of the 4th and 7th generations of *H* was even higher than that of the controls. But after that time there occurred a sharp decline, and with two exceptions, each generation had a lower productivity than the preceding one. The line died out in the 13th generation.

The *Q* series followed in general the same course as the *H* series, but with an average fecundity consistently much lower throughout the experiment. In the first generation grown in the weak culture medium, the *Q* animals produced on the average less than three per cent as many eggs as the control animals. In the next few generations they improved

and reached their maximum egg production in the 5th generation with an average fecundity of about seventy per cent of the record of the controls. From that time on there was a steady decline, each generation having a lower fecundity than the previous one. The maximum depression was reached in the 8th generation with the complete dying out of the line.

Subjecting *Proales sordida* to culture media of reduced strength for

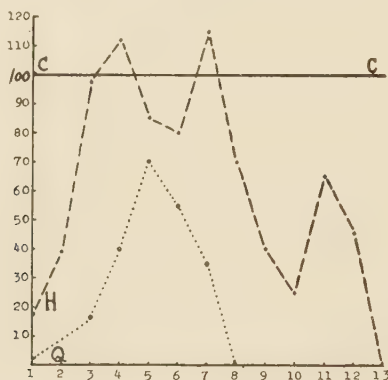


FIG. 4. Experiment I, 1928. Depression curve based on egg production. Horizontal axis represents successive generations; vertical axis the percentages with the record of the controls (*C*) as 100 per cent, and the records of *H* and *Q* plotted with reference to that standard. In *H* the culture medium is half the strength of that in the controls; in *Q*, it is one quarter of that strength.

many successive generations is thus shown to cause a decrease in fecundity similar to that observed in other rotifers in adverse environmental conditions, and similar in many respects to the lowering of fission rate noted in poorly-fed Infusoria.

(b) Longevity.

There is very little in the literature on the effect of adverse conditions on longevity. Most of the environmental studies on the Rotifera present data dealing only with the effect on fecundity, considered as an index of vitality. For this reason the longevity data of these experiments are of special interest. Those for Experiment I are presented graphically in Fig. 5 in the manner already described in the consideration of fecundity. Table III gives a résumé of the records for every generation.

The effect on the length of life of the two underfed series was greater than the effect on fecundity, discussed in the last section; this is especially true of the *H* series. Neither the *Q* series nor the *H* series ever equalled in average length of life the record of the *C* group; and the records of

the three coexisting groups fall in precisely the same order, throughout their history, as that of the strengths of their culture media.

In the *H* group, the first generation lived, on the average, about sixty-five per cent as long as the contemporaneous members of the *C* group. Generations 2 to 7 showed an increase in relative length of life, rising in the 7th generation to about ninety-five per cent of the *C* record. Generation 8 showed a sharp decline which continued with two exceptions, until the 13th generation, when the line died out.

In the *Q* group the average life of the first generation was about

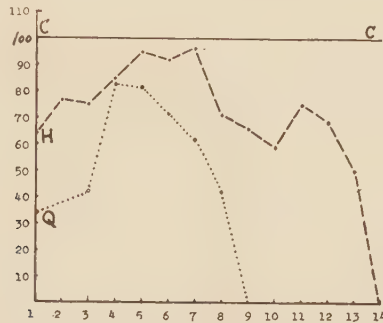


FIG. 5. Experiment I, 1928. Depression curve based on length of life. Horizontal axis represents successive generations; vertical axis represents the percentages with the record of the controls (*C*) as 100 per cent, and the records of *H* and *Q* plotted with reference to that standard.

thirty-five per cent of that of the controls. The next few generations showed an improvement, and in the 4th generation the average longevity of the *Q* line practically equalled that of the 4th generation of the *H* group. From then on, however, there was a rapid decline; the line died out in the 8th generation.

These results are diverse from those obtained on the effect of alcohol by Noyes for *Proales decipiens*, and by Finesinger in most of his experiments on *Distyla inermis*. They did not find that the length of life was appreciably decreased by subjection to alcohol, even though the depressive effect on fecundity was very marked. Also, Finesinger found that in very weak doses of alcohol, both fecundity and longevity were increased. In the experiments here reported, on *Proales sordida* cultivated in inadequate media, both fecundity and longevity were strikingly decreased.

In general, then, we may say that *Proales sordida*, when grown in dilute culture media (quarter or half as strong as the optimum), and under variable temperature conditions, after an initial immediate depression, tends to adjust itself for a few generations and pursue an up-

ward course, but eventually becomes progressively depressed and dies out completely. Individuals grown in a quarter-strength solution have a lower fecundity and longevity than those grown in a half-strength medium, and in turn, individuals grown in a half-strength medium, for the most part, have a lower fecundity and longevity than those grown in a full-strength solution. In other words, the degree of depression and the speed with which it occurs are, to a large extent, dependent on the strength of the culture medium.

III. Recovery.

The question of the heritability of induced modification is of very great interest; it forms the problem of chief concern in these experiments. To test this, some of the progeny of each of the depressed groups were placed in control medium (full-strength) and their suc-

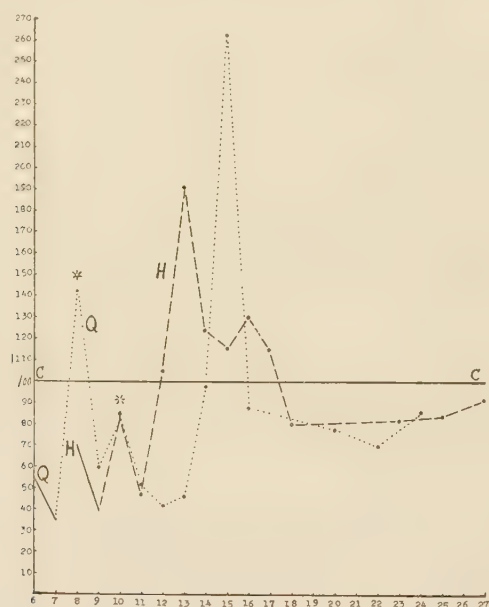


FIG. 6. Experiment I, 1928. Recovery curve based on egg production. Horizontal axis represents successive generations; vertical axis represents the percentages with the record of the controls (*C*) as 100 per cent, and the records of *H* and *Q* plotted with reference to that standard. Asterisks indicate the generations in which return to normal medium occurred.

cessive generations were continuously cultivated in this medium. These "recovery groups" (*HC* and *QC*) were begun with eggs from the ninth generation of *H* and from the seventh generation of *Q*. In the curves showing the records of these groups (Figs. 6 and 7) the first genera-

tions are marked with an asterisk. All computations were made on the percentage basis described above, using the record of the controls as the standard for comparison. On the vertical scale are plotted the percentages; on the horizontal scale the number of generations. The record of the controls is indicated by the solid straight line at 100 per cent. The short solid lines preceding the dashed *HC* line and the dotted *QC* line represent the records of the generations of depressed animals (*H* and *Q*) from which the recovery groups were begun. For the detailed data on fecundity and longevity, and the numbers of animals comprising each generation, consult Table III.

(a) Fecundity.

At the time when the return to normal medium was made, the *H* series had an average fecundity about forty per cent of that of the control animals. As is shown in Fig. 6, the first generation in the normal medium (generation 10, marked with an asterisk) showed a considerable increase in productivity, rising to about eighty-five per cent. The next *HC* generation (generation 11) fell to almost forty-seven per cent again. The third *HC* generation (generation 12) improved markedly, with an average egg production slightly higher than that of the control series; and the next five generations, though showing a considerable fluctuation, maintained an average fecundity consistently above that of the controls. In the 9th generation in normal medium (18th generation of cultivation) the *HC* group had an average fecundity of 80 per cent and stayed at about that level for the remainder of the experiment.

The animals from the *Q* line which were placed in control medium (full-strength) also showed great variation in fecundity. Ten of the twelve *QC* generations had a fecundity lower than that of the controls. The other two (first and eighth *QC* generations) showed a fecundity much higher than that of the controls. The first *QC* generation showed an increase from 36 per cent (the record of the preceding *Q* generation, cultivated in quarter-strength culture medium) to about one hundred and forty-one per cent, exceeding the control record by a wide margin. The eighth *QC* generation (comprising only two animals) rose to 262 per cent, more than doubling the record of the controls.

For a correct evaluation of the significance of these very irregular records of the *HC* and *QC* series, the numbers of animals in the various generations (given in Table III) must be taken into account. With at most two exceptions the early generations of recovery are composed of so few individuals as to render the significance of their data doubtful. As far as they go, they indicate that recovery of egg production is immediate and complete in the first generation grown in normal culture medium.

(b) Longevity.

On the whole, in both test lines, variation in length of life after the return to normal culture medium was much less than variation in egg production. The data are shown in Fig. 7, plotted on the percentage basis as earlier described. The first generations in full-strength culture medium are indicated by asterisks.

The average length of life of the first generation of the *HC* series

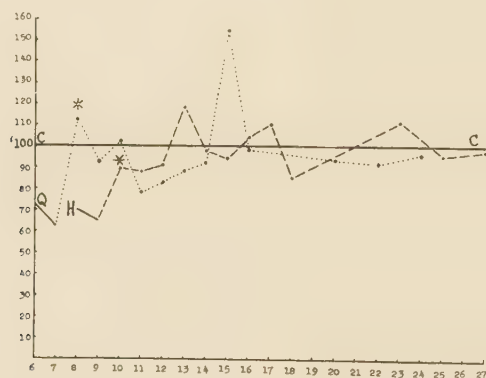


FIG. 7. Experiment I, 1928. Recovery curve based on length of life. Horizontal axis represents successive generations; vertical axis represents the percentages with the record of the controls (*C*) as 100 per cent, and the records of *H* and *Q* plotted with reference to that standard. Asterisks indicate the generations in which the return to normal medium occurred.

was considerably greater than that of the preceding *H* generation. This increase in longevity was continued for several generations, the 4th generation of recovery (13th) exceeding the record of the controls. From that point on, there was considerable variation, the average length of life of the *HC* series fluctuating around the record of the controls. Here, as in the case of fecundity, the fact that the early generations comprised only small numbers of animals makes the significance of their data doubtful.

In the *QC* series, the average length of life of the first generation exceeded the record of the controls, indicating that recovery was immediate and complete. In generations 2 and 3 (9 and 10) the longevity of the *QC* series remained very close to that of the controls; but after that, except for one generation (15th) the average was slightly lower than that for *C*. Here, also, the fact that only one (generation 10) of the first nine generations of recovery in this group was significant from a statistical point of view makes the results unreliable. However, the indication is that the decreased length of life produced by insufficient food is not inherited when the progeny are placed in an adequate environment.

Discussion

The results of these experiments show that the Rotifera do respond to an unfavorable environment as do the Infusoria; they become progressively depressed, show a gradual lowering of vitality, and eventually die out. In the adverse condition used, an inadequate culture medium, successive generations show this graded decline in both fecundity and longevity.

The results bearing on the question of the heritability of induced modifications, however, are not decisive, owing to their great irregularity. It seems probable that this irregularity was largely due to the extreme temperature variation and to the inconsiderable number of animals available in the critical generations. To control the temperature, and in the light of the experience gained to test again the heritability of the depression, a second series of experiments was carried out. An account of these follows.

PART II. EXPERIMENTS OF 1929

In 1929 the experimental work was conducted in a constant temperature room where fluctuations were very slight (20° to 22° C.). Variations from day to day were usually less than half a degree, and omitting the last week of the experiment when considerable fluctuations occurred, the total variation for the summer was less than two degrees.

Preliminary tests were made to find out whether cultivation under uniform temperature necessitated any change in experimental procedure. It was found that under constant temperature conditions rotifers reared in the culture medium used for the controls in the former experiments (15 flakes of oatmeal to 100 cc. spring water) had a very low fecundity and longevity and appeared to be starved. Consequently, solutions of 16, 20, 24 and 32 flakes of oatmeal to 100 cc. of spring water were tested. The 32 flake fluid was found to be the most satisfactory.

In Experiment II, therefore, the following culture media were used:

Control Series	C, full-strength	fluid, 32 flakes oatmeal to 100 cc. spring water
Test	" H, half-strength	" 16 " " " " "
"	" Q, quarter-strength	" 8 " " " " "

In general these media were prepared as earlier described under "Methods," except for the fact that the culture fluid was corked at the end of three hours and placed in the constant temperature room, instead of being corked at the end of twelve hours and subjected to variable temperature. It will be observed that in Experiment II the half-strength and quarter-strength culture media were twice as strong as the corresponding media of Experiment I, and that the fluid for the control series was more than twice as strong as the full-strength medium formerly

used. In all probability, the fact that the culture fluid was exposed to the air for such a short time (three hours) explains the necessity for increasing the number of flakes of oatmeal used.

General Description

This experiment was begun August 11, 1929, with a single series of control animals which were cultivated on slides for three generations before the test groups were started. These test groups, again called *H* and *Q*, were begun with some of the eggs laid by the third generation of this control series. In the 4th generation, therefore, there were three series of animals: the controls (*C*) cultivated in thirty-two flake fluid; the *H* series cultivated in sixteen-flake fluid; and the *Q* series cultivated in eight-flake fluid.

In general, Experiment II was conducted in the same way as Experiment I, dealing again with the effects on fecundity and longevity produced by continuous cultivation for many generations in weak culture media. When the animals had shown a marked decrease in productivity and length of life, the heritability of this environmental modification was tested by placing progeny from both test groups in the control medium of thirty-two flakes, and cultivating them in this full-strength medium for several successive generations.

Analysis of the Data

Since the corresponding generations of all three groups of animals occurred at the same time (except during the last part of the experiment, when the control line was sufficiently in advance of the other two groups to produce one more generation), and since the temperature conditions were satisfactorily controlled, there was no reason for comparing the groups of animals on any basis except that of corresponding generations. The results of the experiment are shown on this basis in Figs. 8 to 11. In these curves the horizontal axis represents the number of generations, using 1, 2, 3 for the preliminary series, then re-numbering 1, 2, 3, etc., beginning with the first generation to comprise three groups of animals. The vertical axis represents either the mean number of eggs or the mean length of life. The data for these curves will be found in Table IV.

I. Depression.

In this experiment, conducted under constant temperature conditions, and with stronger media than those used in Experiment I, the test lines, *H* and *Q*, did not die out with the passage of generations. When the

TABLE IV

Experiment II, 1929—Showing for the successive generations (Gen.) of each of the five series of the second experiment (Control, *H*, *Q*, *HC*, *QC*) the numbers of eggs isolated (No.), the numbers of these that did not hatch (N.H.), and the numbers that hatched (H.); and for the latter the mean numbers of eggs laid and the mean numbers of days lived. Data presented graphically in Figs. 8, 9, 10, 11.

Control Series (full-strength medium)					
Preliminary					
Gen.	No.	N.H.	H.	Mean Eggs	Mean Days
1	24	0	24	20.75	10.00
2	21	0	21	16.95	8.66
3	39	1	38	17.28	8.89
Principal					
1	48	1	47	13.02	7.67
2	52	0	52	12.34	8.17
3	41	3	38	14.00	9.13
4	40	1	39	15.38	9.01
5	40	0	40	13.87	8.17
6	54	3	51	14.31	7.93
7	30	3	27	10.48	7.62
8	34	0	34	9.23	7.26
9	30	2	28	9.53	6.94
10	35	4	31	10.19	6.30
11	29	0	29	8.13	5.79
12	28	0	28	6.46	5.32
13	30	7	23	6.26	6.19
14	39	12	27	9.03	7.35
15	32	10	22	8.59	9.00
<i>H</i> Series (half-strength medium)					
1	50	1	49	9.97	7.34
2	52	1	51	8.25	7.58
3	43	2	41	5.63	6.76
4	39	0	39	6.74	7.55
5	40	4	36	6.97	7.09
6	43	2	41	6.04	6.71
7	51	5	46	5.21	6.71
8	42	4	38	5.63	6.82
9	41	1	40	6.22	6.13
10	39	3	36	4.36	5.97
11	42	3	39	3.82	5.33
12	42	9	33	3.54	6.12
13	36	6	30	5.53	6.71
14	27	4	23	8.52	8.50
<i>HC</i> Series (full-strength medium)					
1(13)	38	4	34	12.50	8.67
2(14)	29	1	28	8.17	8.26

TABLE IV—*Continued*

Gen.	No.	N.H.	H.	Mean Eggs	Mean Days
<i>Q</i> Series (quarter-strength medium)					
1	41	0	41	5.19	5.74
2	38	2	36	2.75	5.13
3	43	7	36	1.33	3.68
4	31	0	31	4.29	6.51
5	51	1	50	5.46	6.59
6	48	4	44	3.00	5.30
7	40	1	39	2.66	5.66
8	40	4	36	1.83	5.00
9	42	3	39	1.64	4.24
10	62	11	51	1.39	4.00
11	63	9	54	2.33	4.93
12	54	5	49	3.51	5.72
13	28	0	28	2.10	4.60
14	34	4	30	1.53	5.05
<i>QC</i> Series (full-strength medium)					
1(9)	24	1	23	9.43	6.04
2(10)	50	9	41	8.85	6.48
3(11)	41	16	25	6.72	6.18
4(12)	39	2	37	12.91	8.29
5(13)	27	4	23	10.39	7.50
6(14)	31	4	27	6.03	7.14

experiment was concluded they had lived fourteen generations and, while showing some depression, they were reproducing well enough to make it seem probable that they would live many more generations, if not indefinitely.

(a) Fecundity.

Figure 8 presents graphically the data on egg production. It shows that in general the records of the three groups of animals ran parallel to each other, the half-strength (*H*) series regularly lower than the control series, and the quarter-strength (*Q*) series regularly lower than the *H* series. At first, the difference between the three groups was very great, but at the end of the experiment this difference was much less, especially between the *H* and *C* series. The control series showed considerable variation, but its average fecundity gradually decreased as the experiment progressed. The *H* line declined rapidly for the first three generations, and from then on continued to show a fairly constant though much less extensive decline until the 13th and 14th generations. In these last

two generations the *H* line showed a marked improvement, almost equaling the average of the controls. The *Q* line likewise declined rapidly when first subjected to the reduced culture medium, showing in the third generation the maximum effect on fecundity, when the average egg production fell to 1.33. The average fecundity stayed consistently low for



FIG. 8. Experiment II, 1929. Depression curve based on egg production. Horizontal axis represents successive generations; vertical axis, mean egg production.

the remainder of the experiment although showing considerable variation. It showed no improvement in its final generations such as the *H* series showed; the 13th generation had an average fecundity of only 2.1 eggs and the 14th generation of only 1.5 eggs. The individuals were small and thin.

Thus in this experiment, as in the former one, decreased food is associated with a decrease in fecundity, the most marked decrease occurring in the first three generations.

(b) Longevity.

The effects on longevity are presented in Fig. 9. The *H* series showed a moderate and gradual decline for eleven generations, but then began to improve; in the 12th, 13th, and 14th generations its average longevity exceeded that of the controls of the corresponding generations.

In the *Q* line the effect of the adverse condition was more marked,

and for the first few generations the average length of life declined rapidly, reaching its lowest point in the third generation. From that time on the *Q* line showed considerable variation but remained consistently below the other two groups except in the 12th generation, when the

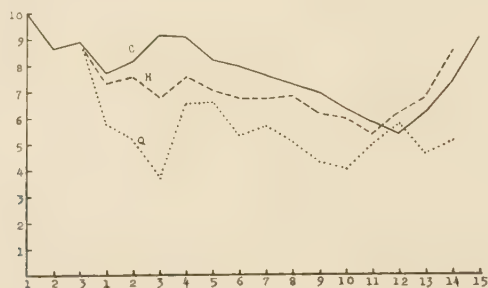


FIG. 9. Experiment II, 1929. Depression curve based on length of life. Horizontal axis represents successive generations; vertical axis, mean length of life in days.

average length of life was slightly above that of *C*, but did not equal that of *H*.

II. Recovery.

The *H* recovery series was not begun until very late in the experiment. We wished to test progeny from the most depressed generation and for that reason delayed beginning this series in anticipation of further depression in *H*, which did not occur. The *HC* series was finally started with eggs from the 12th *H* generation, a short time before the experiment terminated, and so was carried through only two generations. The *Q* recovery series was begun with eggs from the 8th generation in the quarter-strength medium. This *QC* line was continued in normal medium for six generations, when the experiment ended. The data on these *HC* and *QC* lines is presented in Figs. 10 and 11. Asterisks indicate the generations where the return to normal medium occurred.

(a) Fecundity.

While the *H* line in the 12th generation had an average egg production of 3.54, their progeny which were returned to the normal medium had an average of 12.5, exceeding the average of the controls by 6.24 eggs. The second (the last) *HC* generation fell to an average slightly below that of the controls.

The *Q-QC* series increased from an average of 1.83 eggs in the 8th generation in the quarter-strength medium to 9.43 eggs in the first generation in control medium, practically equalling the controls of that gen-

eration. They remained slightly below the controls for two generations and then surpassed them by an enormous margin in the 4th generation (twelfth under cultivation). After this they again declined to a point below the controls. The records of these two lines certainly provide

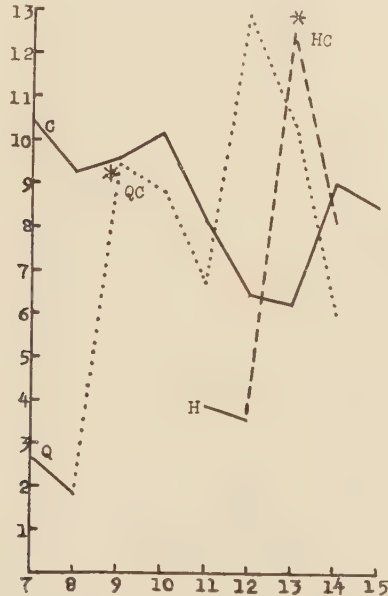


FIG. 10. Experiment II, 1929. Recovery curve based on egg production. Horizontal axis represents successive generations; vertical axis, mean egg production. Asterisks indicate generations in which return to 100 per cent medium occurred.

conclusive evidence that the effect of insufficient food on fecundity is not inherited.

(b) Longevity.

Figure 11 clearly shows that animals from both the test series, when placed in full-strength culture medium, recovered a normal longevity at once, their records even exceeding that of the control animals. The record of the first *HC* generation exceeded by the same amount the record of their parents (the last *H* generation) and the record of the corresponding generation of the *C* series, and fell only slightly in their second and last generation.

The longevity of the first generation of the *QC* line, grown in the full-strength medium, almost equalled the record of the controls; the four generations following exceeded the controls, and the sixth (the last) generation was only very slightly below the controls.

What has been said concerning the inheritance of decreased fecundity is likewise true for longevity. Animals whose length of life has been altered by long cultivation under adverse conditions produce progeny that show a normal longevity when grown in a favorable environment. Depression produced in this rotifer by the action of an inadequate culture medium lasts only as long as the stimulus which produces the depression is present. This result differs a little from the results of Whit-

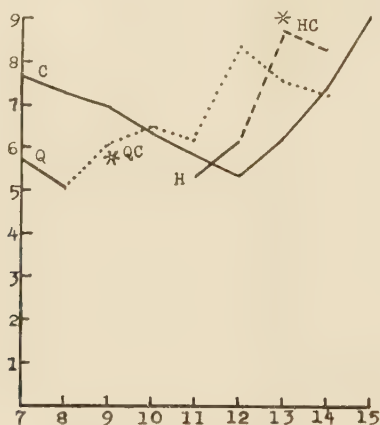


FIG. 11. Experiment II, 1929. Recovery curve based on length of life. Horizontal axis represents successive generations; vertical axis, mean length of life in days. Asterisks indicate generations in which return to 100 per cent medium occurred.

ney (1912) and Noyes (1922), who found that the depression produced by the action of alcohol persisted for two generations after removal to a favorable medium.

Mechanism of the Effect

As to the precise way in which the environment operates to produce depression, we have no evidence. It may be that in a medium deficient in food, the rotifer grows at the expense of its own productivity; that in the ordinary metabolic processes it uses up part or all of the yolk stored in the vitellarium, making the production of many eggs impossible, thus bringing about a depression in fecundity. This process may be correlated with the length of life of the organism to the extent that when all the stored yolk has been used up in metabolism, the animal has no more reserve on which to depend and consequently dies, thus bringing about a lowering in longevity. Or, on the other hand, in such a weak food medium the animal may use all the food it receives for its own body metabolism and not have enough left to produce yolk for many eggs, causing a reduction in fecundity but not in longevity, the animals

having sufficient vitality to continue living for the usual length of time if they do not use part of their energy in egg production.

It is possible that the amount of stored yolk is reduced in ill-fed animals, and that the eggs are smaller. If this were a cumulative process, the eggs of consecutive starved generations becoming progressively smaller, containing less and less yolk, the lines would eventually die out. Measurements need to be made to determine whether there is any size difference between the eggs of normal parents and the eggs of depressed parents. As far as we could determine by superficial observation, the eggs of all generations of all three groups were normal in every way.

No measurements were made on the animals in our experiments, but some of the observations on comparative size and general appearance are of interest. In the second experiment, particularly, the difference in appearance between animals belonging to different lines was very striking. The controls were large and healthy in appearance from the first; the animals of the *H* series very soon became slightly smaller than the controls, and quite transparent; the *Q* individuals were extremely small and thin. For several generations one could recognize at once to what line an animal belonged merely by its size and type. As the experiment proceeded, however, the *H* and *Q* animals improved in appearance to such an extent that in the later generations they seemed as healthy and normal as the control animals. In the last two generations some of the control animals looked bloated and heavy, and died containing dark masses of material, and often one or two or even three well-developed eggs which they had been unable to lay.

In both of the experiments, animals were observed with internal eggs which they were unable to eject; in numerous cases the egg developed within the female and hatched within her body after her death. Some of these young finally escaped from the parental corpses and lived thereafter as normal individuals, apparently not differing from animals that had hatched normally. A single instance was observed where two such young hatched out within the same female and later became liberated. This behavior was not confined to one group or one generation, but instances were observed in *C*, *H*, and *Q*, in the later generations of both experiments.

SUMMARY AND CONCLUSIONS

This paper is a study of depression and recovery in the parthenogenetic rotifer, *Proales sordida* Gosse, showing the response of the organism to various dilutions of culture medium. All the animals used were members of a single clone and hence were genetically alike. Oat-meal infusions of half and quarter-strengths, as compared with the full-strength medium used for the control animals, were employed. The in-

vestigation consisted of two series of experiments conducted separately one year apart, together with some supplementary procedures. It was found that with continuous parthenogenetic reproduction, rotifers in the reduced strengths of culture media become depressed, decline in vitality with the passage of generations, showing a much lowered fecundity and longevity. Under conditions of variable temperature this decline is cumulative and results in the ultimate death of the lines. Under constant temperature conditions the animals show a cumulative decline in the first few generations, reaching a low level of fecundity and longevity, where they remain without further depression. The degree of depression and the speed with which it occurs depends on the strength of the medium employed. This depression produced by means of an environmental agent is similar in many ways to that produced by the action of adverse conditions in various Infusoria and in other rotifers. However, in contrast to the results obtained by others in studies of the Rotifera, longevity as well as fecundity is affected by the environmental agent.

The progeny of the depressed animals recover their normal fecundity and longevity at once in the first generation placed in a favorable medium. Apparently the food deficiency does not affect the germ cells so as to produce a heritable modification.

The protozoan constitution seems to be extremely sensitive to environmental modifications and becomes changed to such an extent that the alterations are passed on to the progeny of later generations. Some of these changes are adaptive, so as to cause an increase in resistance to certain agents, while others (such as those resulting from food insufficiency) are degenerative and result ultimately in the extinction of the race. The situation in the Rotifera, as apparently in the other Metazoa, is quite different. Though in Rotifera it is possible to produce and maintain depression for a long period of time, and though the depression becomes greater in later generations, it disappears at once, or within one or two generations, upon restoration to normal conditions. The complete agreement in this respect of the results of the present study with those of Whitney, Noyes, and Finesinger on the injurious effects of alcohol goes far to establish this as a definitive conclusion for the Rotifera. Injurious conditions acting on the individuals of many successive generations and producing a progressive decline, do not alter the germinal materials carried by these individuals.

It is possible that this difference between the Rotifera and the Protozoa may be related to the differences in the details of their reproductive processes. In the Protozoa the body divides into two equal parts; each part becomes a new individual. In *Proales*, as in most Metazoa, a smaller piece separates from a larger. The larger piece is called the

parent; the smaller the egg. This smaller piece goes through a twenty-four-hour-long process of development, including numerous cell divisions, into a new *Proales* individual. It does not seem obvious *a priori* that this difference in detail must result in diverse genetic behavior. These experiments on the heritability of an environmental effect indicate that they do.

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VITA

Helen Berenice Smith was born June 28, 1901, in St. Paul, Minnesota. Her grammar school education and her first year and a half of high school were completed in that city. She finished her high school training in Oskaloosa, Iowa, in 1919, and spent the following four years teaching in the consolidated schools of rural Iowa. She received her B.S. degree from Penn College in June, 1926, having majored in biology, and was awarded the Bryn Mawr Scholarship. Her first year of graduate study (1926-1927) was spent at Bryn Mawr College, working under the direction of Dr. D. H. Tennent. In collaboration with Miss Ruth A. Miller, she carried on an investigation, the results of which were incorporated in a paper entitled "Observations on the Formation of the Egg of *Echinometra lucunter*," which is as yet unpublished. The following three years (1927-1930) were spent in the Department of Zoology at The Johns Hopkins University working mainly under the direction of Professor H. S. Jennings. The summers of 1928 and 1929 were spent in research in genetics at the Marine Biological Laboratory, Woods Hole, Massachusetts. In February, 1930, she was appointed Research Assistant in the Department of Genetics of the Carnegie Institution, and having received leave of absence from the University, she has worked in the laboratory of the Carnegie Institution from that time till June, 1930.

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